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PRESERVING VIBRIOS BY LYOPHILIZATION

[Following is the translation of an article by A. V. Golikov, Doctor of Veterinary Sciences, Tselinogradskaya Scientific-Research Veterinary Station, and V. S. Polyakova, Scientific Worker at the Kazakhskiy Scientific-Research Veterinary Institute, published in the Russian-language periodical Veterinariya (Veterinary Affairs), No 9, 1968, pages 108-109.]

It is difficult to preserve vibrios under laboratory conditions. For example, on semiliquid meat-peptone liver agar (MPPA) cultures of vibrios must be transplanted every 10-12 days. However, as a rule the strains dissociate and coccoid forms are formed. Antigenic, agglutinogenic, and pathogenic properties are weakened. Often the strains of vibrios are lost altogether.

Mund (1956) and I. G. Levina (1964) pointed out the possibility of preserving vibrios (up to 9 months) by the method of lyophilic drying; V. P. Parusov and N. Savov reported of the survival of vibrios in a frozen state at -79° for up to 5 months and at -28° for up to 15 days.

For the purpose of studying the possibility of prolonged preservation of strains of vibrios under laboratory conditions at the experimental-technological laboratory of the Kazakhskiy NIVI a check was made of the lyophilic drying of four strains of vibrios, of which two were related to the type *Vibrio fetus venerealis*, one to *V. fetus intestinalis*, and one to the type *V. bubulus*. All the strains were preliminarily checked for cultural-morphological, biochemical, and pathogenic properties.

The cultures of vibrios were incubated for three days at a temperature of 37° on semiliquid MPPA (pH 7.2) in flasks with a capacity of 100 ml.

After incubation two-thirds of the underlying layer of semiliquid agar, which did not contain vibrios, was drawn off, which made it possible to create a concentration of vibrios of up to 2 billion microbial cells in 1 ml of medium. Then the culture of vibrios was mixed with protective medium in a ratio of 1:1 and poured into ampoules of 1 ml each.

Duration of preservation of vibrios in a dried state

Protective medium	Residual humidity (%)	Time (months) and results of seeding				
		3	6	12	18	24
1% peptone	3.9	+	-	-	-	-
Horse serum	2.2	+	+	-	-	-
Amniotic fluid of cattle	10.4	-	-	-	-	-
Amniotic fluid of sheep	9	-	-	-	-	-
Amino peptide-2	4.8	+	+	+	+	+
Medium No 3	7.3	-	-	-	-	-
Separated milk	3	+	+	+	+	+
5% gelatose + 10% saccharose	3.5	+	+	+	+	+
1.5% gelatin	9.2	+	+	-	-	-
1.5% gelatin + 10% saccharose	6.7	+	+	+	+	+

Ten media were tested as protective ingredients: 1% peptone, normal horse serum, amniotic fluid of cattle, amniotic fluid of sheep, amino peptide-2, amino acid medium No 3, separated milk, 5% gelatose with 10% saccharose, 1.5% gelatin, and 1.5% gelatin with 10% saccharose.

After combining with the protective media the cultures of vibrios were frozen for 8 hours at -50° and then dried for 24 hours under a vacuum from 100 to 50 microns with a temperature of from -10° to $+25^{\circ}$ in the drying chamber. After drying the ampoules were checked for airtight sealing and then stored at room temperature.

Control seedings on semiliquid MPPA were made immediately after drying and then in 3, 6, 12, 18, and 24 hours. Simultaneously a check was made of the cultural-morphological, biochemical, and pathogenic properties of the incubated cultures of vibrios (see table).

An analysis of the results shows that amniotic fluid of cattle and sheep and also medium No 3 turned out to be unsuitable as protective media for the drying of vibrios. Strains of vibrios were preserved for 24 months in the 5% gelatose with 10% saccharose, amino peptide-2, separated milk, and 1.5% gelatin with 10% saccharose media.

The cultural-morphological, biochemical, and pathogenic properties of the dried strains remained at the initial level. The experiment for clearing up the question of survival of vibrios, which is calculated for three years, will be continued.